

CRISPR/Cas9 genome editing

 Morayma M. Temoche-Diaz

Updated date: Nov 23, 2019

 An abbreviated version of this protocol was published in eLIFE in Aug 2019

Distinct mechanisms of microRNA sorting into cancer cell-derived extracellular vesicle subtypes

DOI: 10.7554/eLife.47544

Detailed protocol

A pX330-based plasmid expressing Venus fluorescent protein (Shurtleff et al., 2016) was used to clone the gRNAs targeting Rab27a and Rab35. Two CRISPR guide RNAs targeting each gene were selected using the CRISPR design tool (Hsu et al., 2013). For Rab27a, gRNAs targeting exon 4 and exon five were selected. For Rab35 both gRNAs targeted exon 1. Both gRNAs targeting each gene were cloned simultaneously in the pX330-Venus plasmid according to the PrecisionX Multiplex gRNA Kit instructions (SBI). MDA-MB-231 cells at 25% confluency were transfected for 48 hr, and then, they were trypsinized and sorted for single Venus-positive cells in a 96 well plate using a BD Influx cell sorter. Wells containing clones were allowed to expand (30 clones for Rab27a and 25 for Rab35) and knockouts for each gene were confirmed by immunoblots.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Temoche-Diaz, M. M.(2019). CRISPR/Cas9 genome editing. Bio-protocol Preprint. bio-protocol.org/prep44.
2. Temoche-Diaz, M. M., Shurtleff, M. J., Nottingham, R. M., Yao, J., Fadadu, R. P., Lambowitz, A. M. and Schekman, R.(2019). Distinct mechanisms of microRNA sorting into cancer cell-derived extracellular vesicle subtypes. eLIFE. DOI: [10.7554/eLife.47544](https://doi.org/10.7554/eLife.47544)

Copyright: Content may be subjected to copyright.